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A Case Study on Preliminary Phytochemical Screening Successive Solvent Extraction

Mohd. Abdul Samee

Research Scholar - Pharmacy, Shri JJT University, Jhunjhunu, Rajasthan, India.

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ABSTRACT

The crude drug was dried when it is subjected for extraction using another solvent. The previous solvent should be removed completely and powder should be dried totally. It prevents the mixing of the previous solvent into another solvent. After the complete extraction, solvent were evaporated on rotary evaporator and solvent was removed, the extract thus obtained with 95% alcoholic solvent was measured. The extract was stored in desiccator. Preliminary qualitative tests going with procedures were acclimated to tests for acknowledgment of various compound constituents in each distills.

Keywords: Extract; Phytochemical; Carbohydrates; Glycosides; Vitamin C.

Introduction

Preliminary Phytochemical screeningSuccessive solvent extraction

Choice of solvent depends on nature of plant material & components to be isolated. Latter is also particularly useful for stabilizing fresh leaves by dropping them into boiling solvent (Harborne JB, 2007).

Soxhlet extractor is the simplest way for preparation of extracts of crude drugs. Pure solvent is used in this technique. The crude drug used for extraction is kept in a 'thimble' made of cloth or cellulose in middle portion of the Soxhlet apparatus. The solvent used for extraction is kept in the lower portion and a condenser is connected above the middle compartment.

The solvent is added in Round Bottom Flask and heated to boil to formvapours. The hot solvent passes through the crude drug and extraction takes place.The extract gets deposited in the lower portion of middle compartment. The same process was repeated till complete extraction of crude drug takes place.

In this technique of extraction the extract get collected in the lower RBF, gradually becomes concentrated.

Different solvents with increasing polarity can be used for the continuous total extraction, e.g. benzene, Hexane, pet ether, chloroform, methanol, ethanol and water.

The dried powder of stems used for extraction procedure was sieved through 60-120 mesh to separate fine and course powder. This course powder was utilized for further extraction. The extraction was performed by using continuous hot extraction using Soxhlet apparatus till removal of all constituents takes place.

Weighed 2.5 kg of plant material subjected to solvent extraction process using Soxhlet extraction unit. Preferred suitable solvents were Petroleum ether, chloroform, ethanol and chloroform water used as solvent by increasing the polarity (Figure 1).

This method gives solubility of chemical constituents from sufficient quantity of crude drug when it is done by specific solvent. When extraction done with different solvents and for different crude drugs.

It gives variable results about phyto-constituents. The availability of different constituents in specific solvent mainly depends upon nature of drug and solvent used for extraction. (Mukherjee, 2002) After the extraction process % yield of the extract determined as follows:

$$Percentage\ yield \quad \%w/w = \frac{Wt.of\ extract}{Wt.of\ the\ sample} \times 100 \qquad (1)$$

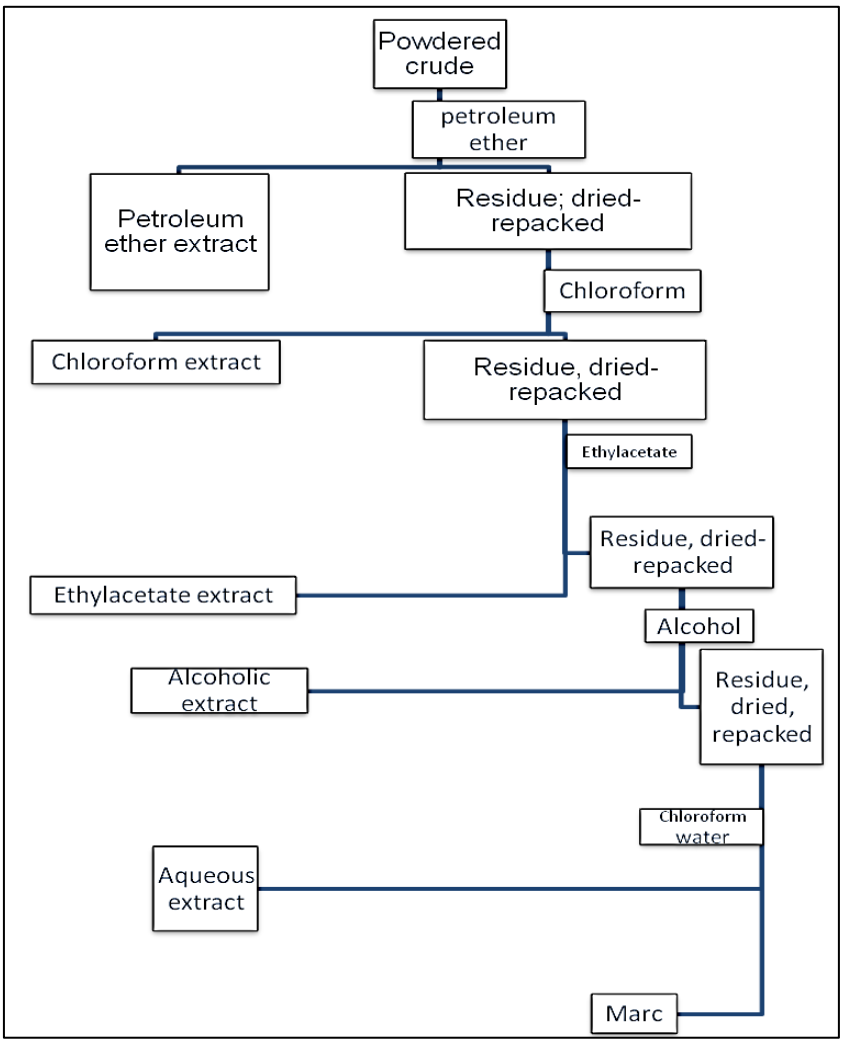

Figure 1. Solvent Extraction Scheme

Preliminary qualitative chemical examination

Preliminary Qualitative Chemical Investigation of Extracts (Harborne, 1988; Kokate, 2002) Subjective invention examination is performed on Petroleum ether (40-600C), Alcohol & Fresh Aqueous concentrates.

For each think & division test plan (test course of action) was set up as 1%w/v of each focus/partition in its individual dissolvable & same was used for preliminary subjective examination.

Tests for Carbohydrates

Take 2-3 ml of think & incorporate some drops ofnaphthol course of action, shake & incorporate conc.H_2S0_4 from sides of tube. Ring (Violet) at convergence between to liquids is occurring.

For Reducing Sugars

Take 1 ml of Fehling course of action A & B, both are mixed & incorporate ascent to volume of test game plan. Warmed in percolating water shower for 5-10 min. yellow as piece red hurry is viewed.

Warmed in gurgling water shower for 5-10 min. yellow, green or red endless supply of decreasing sugar appear in test course of action.

Greenish Blue/purplish or upper layer get chance to be unmistakably greenish blue & lower layer get opportunity to be particularly purplish was viewed. It shows proximity of glucose & fructose

Tests for proteins

Biuret test: Add 3 ml of test game plan in 4% NaOH game plan & 1% $CuSO_4$ course of action. Violet or pink shading was viewed.

Millon's test: Add 3 ml of test course of action with 5 ml of Millon's reagent, white hurry was viewed. Warmed quicken turns piece red or hurry separates giving red shading was viewed.

Xanthoprotein test (tyrosine/tryptophan): Mixed ml of test course of action with 1 ml conc. H_2SO_4 watched white hurry.

Test for protein containing Sulphur: Mix 5 ml of test course of action with 2 ml of 40% NaOH & 2 drops of 10% lead acidic corrosive determination game plan. Plan was percolated it turns dull & tannish.

Precipitation test: test game plan give white colloidal quicken with taking after reagents i) Absolute alcohol ii) 5% $HgCl_2$ Solution iii) 5% $CuSO_4$ solution iv) 5% lead acetate solution iv) 5% ammonium sulphate.

Test for steroids

Salkowski reaction: Take 2 ml of concentration, mix it with 2 ml of chloroform and 2 ml of concentrated H2SO4, and see what happens when the chloroform layer becomes red and the destructive layer fluoresces.

Libermann Burchad Test: 2 ml of thought containing chloroform, 1–2 ml of acidic anhydride, and 2 drops of concentrated H2SO4 from the test tube's side should be added. At first, red shading was seen, then blue, and finally green.

Libermann's test: 3 ml of acidic anhydride were combined with 3 ml of focus. heated and chilled. then include concentrated H2SO4. The blue shading was seen.

Test for Amino Acids

Ninhydrin test (General test): Take 3 ml of test game plan & 3 drops of 5% Ninhydrin course of action & warmed on water shower for 10 min. Purple ol to some degree blue shading was viewed.

Test for Tyrosine: Take 3 ml of test plan & 3 drops of Millon's reagent. Dull red shading course of action was viewed.

Test for tryptophan: To 3 ml T.S. included few drops glyoxalin destructive & concentrated H_2SO_4. Reddish violet

ring at crossing point of two layers was viewed.

Test for cysteine: To 5ml T.S incorporate few drops 40% NaOH & 10% lead acidic corrosive determination game plan. Bubble dim empower of lead sulfate is encircled.

Tests for Glycosides

General test for Glycosides

Part A: 200 mg of prescription think are taken & expelled. By then move wastaken in test tube contains 5 ml of debilitate 10% H_2SO_4 on water shower at 1000 C for 2 min. Observe measureof red hurry encircled & differentiate & that molded to constrained degree B.

Part B: 200 mg of drug focus are taken & removed. By then move was takenin test tube contains 5 ml of debilitate 10% H_2SO_4. In wake of percolating incorporate volume of NaOH used as part of above test.

Differentiate & measure of urge confined to some degree B with that of formed to some degree A.

If rush to restricted degree is more imperative than to some degree B then glycoside may be accessible. Since part B address measure of free diminishing sugar already present in harsh tunneled, whereas area address free reducing sugar notwithstanding those happened on destructive hydrolysison any sides in grungy medicine.

Extract is tested for free sugar after aggregate ejection of free sugars, focus is hydrolyzed with mineral destructive & short time later strove for glycone & glycone moieties.

Raymond's test: Test course of action, treated with Dinitro benzene in hot methanolic solvent base gives violet shading.

Legal test: Treat expels with pyridine & incorporate fundamental sodium nitroprusside plan, dim red shading appears.

Bromine water test: Test course of action, treated with Br water yieldsyellow ppt.

Chemical test for specific Glycosides

Tests for Cardiac Glycosides

Baljet Test: Test game plan reacted with sodium picrate, yellow to orangeshading was viewed.

Legal Test (For cardenolides): To test game plans incorporate 1 ml of pyridine & 1 ml of sodium nitroprusside, pink to red shading was viewed.

Test for deoxysugars (Killer Killiani test): To focus incorporate chilly acidic destructive, 1 drop of 5% $Fecl_3$ & conc.H_2SO_4 looked for reddish chestnut colorant crossing point of two liquids & upper layers shows to some degree blue green shading.

Libennann's test (For bufadienolides): Mixed 3 ml of acidic anhydride with same measure of focus. Warmed & cooled. Incorporate conc. conc.H_2SO_4 & blue shading is viewed.

Tests for Saponin Glycosides (Brain, 1975; Treasy, 1995).

Foam test: Drug focus was mixed with water & shake vivaciously. Persistent foam was viewed.

Hemolytic test: On slide, take test course of action & one drop of blood, Hemolytic zone was viewed.

Test for Flavonoids

Add little measure of test course of action in lead acidic corrosive determination game plan, yellow shaded was viewed.

Shinoda test: To focus, incorporate 95% ethanol in conc. HCl & 0.5 g ofmg turnings. Pink shading was viewed.

To test course of action, incorporate NaOH game plan yellow toned was watched, which was decolorized after development of destructive.

Add ferric chloride course of action in test game plan, extraordinary green shading was viewed.

Tests for Alkaloids: To vanished petroleum ether, watery & alcoholic think, incorporate debilitate hydrochloric destructive game plan & channel, filtrate is used for taking after tests.

Dragendroff's test: Take 2-3 ml of filtrate course of action, incorporate Dragendroff's reagent, orange cocoa quicken was viewed.

Mayer's test: Add 2-3 ml of filtrate in Mayer's reagent looked for hurry.

Hager's test: incorporate 2-3 ml of filtrate in Hager's legitimate, yellowhurry was viewed.

Wagner's test: Add Wagner's reagent in 2-3 ml of filtrate, ruddy chestnutenergize was viewed.

Test for Tannins & phenolic compounds: With 2-3 ml of test course of action, watched taking after color reactions.

5% Fecl3 course of action: Deep blue dull tinted

Lead acidic corrosive induction course of action: White quicken.

Gelatin course of action: White quicken.

Acetic destructive course of action: Red shading game plan.

Potassium dichromate: - Red quicken.

Dilute iodine course of action: Transient red shading.

Dilute nitic destructive course of action: Reddish to yellow shading.

Test for Vitamin C

Incorporate 0.6 ml of hydrochloric destructive (obsession) drop savvy & blend, yellow shading turns blue.

To 2 ml of 2% w/v course of action, incorporate 2 ml of water, 0.1 gm of sod.; significant violet shading made. Incorporate 5ml of 1M sulphuric destructive, shading vanishes.

Tests for volatile oils: Volatile oil from hydro-distillated leaves used for following tests:

Odour- volatile oils have specific odour.

Filter paper stain test.

Tests for fats & oils

Add drop of Sudan Red III reagent on thick fragment of leaf/sedate. Taking after 2 minutes was with half alcohol. Mount in glycerin red oil globules saw under in amplifying focal point.

Solubility test-oils are ether, benzene & chloroform soluble & 90% ethanol& water insoluble.

Filter paper stain test-filter paper gets permanently stained with oils.

Declarations

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The study has not received any funds from any organization.

Competing Interests Statement

The author has declared no competing interests.

Consent for Publication

The author declares that he consented to the publication of this study.

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